

1 BIODEGRADABLE BLOCK COPOLYMERS

2 WITH MODIFIABLE SURFACE

3 Biodegradable Block Copolymers with Modifiable Surface

4
5 The invention

6
7 BACKGROUND

8
9 Technical Field

10 The disclosure relates to block copolymers with a hydrophobic biodegradable
11 component and a hydrophilic biocompatible component, which permit the selective
12 binding of surface-modifying substances and at the same time can suppress the non-
13 selective adhesion of unwanted substances, and to shaped bodies produced therefrom.

14
15 Such block copolymers are particularly suitable as carriers for cells for tissue
16 culture, as carriers for active substances such as medications, in particular for
17 controlled release (drug delivery system) and for targeted administration of active
18 substances (drug targeting).

19
20 Related Art

21 Biomaterials, which include the block copolymers according to the
22 invention disclosure, play a dominant role in a range of medical applications. The term
23 biomaterials relates to substances which assume a specific function in human or
24 animal body as substitute substances for endogenous materials. Examples of this are
25 metals or polymers, such as, for example, those used in total endoprosthesis in the
26 region of the hip joint, for example. A disadvantage of many biomaterials, which are
27 only used temporarily in the body, such as pins or plates in the surgical field, for
28 example, is that they have to be removed after application. For this reason, at the

1 beginning of the seventies an intensive search was started for biodegradable materials
2 which degrade into fragments tolerated by the body during the application.

3
4 The term "biodegradable" means that the biological system, into which the
5 material is introduced, contributes to its degradation [Vert, M et al "Degradable
6 Polymers and Plastics" Redwood Press Ltd. (1992) 73-92]. Those particularly worthy
7 of note are biodegradable polymer materials which degrade into oligomers or
8 monomers. Surgical suture material or degradable carriers of medicinal agents are
9 mentioned as examples of their application.

10
11 The successful application of biodegradable polymers has led to an intensive
12 search for new synthetic materials, from which a plurality of different polymer classes
13 resulted, such as poly(a-hydroxyesters), poly(b-hydroxyesters), polyanhydrides,
14 polycyanoacrylates and many others [Göpferich, A. (1997) 451-472; Göpferich, A.:
15 Biomaterials 17 (1996a) 103-114; Göpferich, A. Eur. J. Pharm. Biopharm. 42 (1996b)
16 1-11].

17
18 A particular characteristic of these polymers is their low solubility in aqueous
19 media, which only improves through polymer chain degradation, i.e. hydrolysis to
20 lower-molecular oligomers or monomers, and thus leads to erosion of these materials.

21
22 Besides the development of synthetic biodegradable polymers, an intensive
23 search was instigated at the same time for natural polymers, which have similar
24 properties. Examples of these are collagens, hyaluronic acid, alginate and cellulose
25 derivatives [Park, K. et al: Biodegradable Hydrogels for Drug Delivery (1993)]. With
26 these substances, it is accepted to some extent that they have an increased water
27 solubility. To lower the water solubility, natural polymers are often chemically
28 modified, e.g. by etherification and esterification of functional groups in the polymer

1 chain or by cross-linkage of individual strands. ¶By way of example, the cross-linkage
2 of collagens, gelatine or alginate are mentioned here.

3
4
5 Various biodegradable polymers differ above all by the rate of polymer chain
6 degradation and erosion. This is important for many applications, in which the
7 polymer chain degradation must extend over a defined time period, such as in the case
8 of release of medicinal agents, for example.

9
10 It is essential for the medicinal application of synthetic, part-synthetic and
11 natural biodegradable polymers that they are compatible with the biological system
12 into which they are introduced. ¶For applications in human or animal organisms,
13 individual structural elements, such as oligomers or monomers, must not be toxic and
14 the polymers may trigger, at most, a moderate inflammatory reaction in the tissue.

15
16 The above-mentioned biodegradable polymers are currently used for the
17 controlled release of medicinal agents (drug delivery) [Göpferich, A. Eur. J. Pharm.
18 Biopharm. 42 (1996b) 1-11] and as carriers for cells (tissue engineering) [Langer, R
19 and Vacanti, J.P. Science 260 (1993) 920-926].

20
21 As part of the drug delivery, biodegradable polymers release medicinal agents
22 in a controlled manner by diffusion, erosion, swelling or osmotic effects.

23
24 In the field of tissue engineering, biodegradable polymers as used as porous
25 "sponges"; for example, on which cells can adhere, proliferate and be differentiated.
26 While the cells develop to a tissue band, the polymer carrier degrades and a tissue
27 results which may be transplanted into the human or animal body.

28

1 Examples of tissues currently produced in this way are, inter alia, cartilage,
2 bone, fatty tissue and vessels.

3
4 The application of biodegradable polymers in the fields of tissue engineering
5 and drug delivery set particular requirements for these materials.

6
7 Besides the already mentioned biocompatibility of the polymers and their
8 degradation products, these applications set particular requirements for the surface
9 properties of the polymers.

10
11 Some examples from the field of drug delivery shall be named firstly:

12
13 1. An adsorption of molecules (for example, medicinal agents, proteins and
14 peptides) onto the polymer surfaces is frequently observed. ¶This can result in the
15 biodegradable medicinal agent carrier not releasing its dosage to the desired extent
16 and not with the desired kinetics. ¶In an extreme case, this can also lead to inactivation
17 of the active substance.¶ The adsorption of active substances is therefore undesirable
18 in many cases and must be suppressed.

19
20 2. The compatibility of a biodegradable polymer is greatly dependent on its
21 surface properties. Hence, these polymers in the form of particles in the micrometer
22 and nanometre range are recognized by cells of the immune system such as
23 macrophages, for example, after absorption of endogenous proteins, and subsequently
24 phagocytised.

25
26 It is therefore necessary to examine the surface properties of small particles as
27 parenteral forms of medicines for their successful use.

28
29

3. Biodegradable nanoparticles have long been sought to use for the targeted administration of substances to specific tissue (for example, tumours or central nervous system) (drug targeting). It has been found in this case that endogenous proteins which are adsorbed on the particle surfaces are responsible for where these particles are transported. [Juliano, R.L.: Adv. Drug Delivery Rev.2 (1988) 31-54]. Hitherto it has only been conditionally possible to achieve a targeted adsorption of these proteins onto the particles. Polymers which allow the targeted modification of their surfaces by simple means are therefore advantageous.

The surface properties of biodegradable polymers also play an important role in the field of tissue engineering:

1. The interactions between cells and polymer determine cell growth and cell differentiation. Natural anchorage mechanisms of the cells are responsible for adhesion of the cells to the polymer surfaces. Proteins such as integrins, for example, allow cells to adhere to specific amino acid sequences. The adhesion to biodegradable polymers occurs as a result of proteins from body fluids or cell culture media adsorbing non-specifically to the polymer surfaces and, in turn, the cells themselves adhering to the corresponding amino acid sequences of the proteins. This non-specific adsorption of proteins causes a plurality of different cells to adhere to the surface. This is above all disadvantageous if a specific cell type is to be adhered to the biodegradable polymer. It is therefore desirable to examine the adsorption of proteins and peptides.

2. The amino acid sequences to which cells adhere are often specific for a cell type, i.e. if the surface of a polymer is coated with a cell-specific sequence, then this cell type preferably adheres.

1 3. The membrane of a cell carries a series of receptors, in which case the
2 behaviour of the cell can be influenced via these receptors. Therefore, if corresponding
3 "signal substances" such as hormones, growth factors or cytokines, for example, are
4 located on the surface of polymers, to which the receptors can bind, the behaviour of
5 the cells adhering thereto via the receptors may be influenced via these
6 correspondingly coated polymer surfaces.

7
8 The above-mentioned examples show the importance of the surface properties
9 of a biodegradable polymer or the importance of the possibility of selective
10 modification of these surfaces for successful application of the polymer.

11 The modification of surface properties of biodegradable polymers has been the aim
12 of intensive research for some years.

13
14 The first attempts at producing biodegradable polymers with modifiable
15 surfaces started from incorporating monomers such as lysin, for example, which
16 contain a functional group to which the molecules can adhere, into the polymer chain
17 of poly(a-hydroxyesters), e.g. polylactide, [Barrera, D.A. "Synthesis and
18 Characterization of a Novel Biodegradable Polymer - Poly(lactic acid-co-lysin)" 1993,
19 Massachusetts Institute of Technology, PHD Thesis].

20
21 A disadvantage of these polymers is that the functional groups, in this case
22 amino groups, are only accessed in the surface with difficulty. In order to improve
23 this, oligopeptides were adhered to these functional groups in order to facilitate the
24 binding of new chemical bonds.

25
26 A disadvantage is that the non-specific adsorption of unwanted proteins and
27 peptides occurs in the polymer obtained.

28
29

1 This led to new developments in order to obtain a more broadly applicable
2 system [Patel, N., Padera, R., Sanders, G.H., Cannizzaro, S.M., Davies, M.C., Langer,
3 R., Roberts, C.J. Tendler, S.J., Williams, P.M. and Shakesheff, K.M. "Spatially
4 controlled Tissue Engineering on Biodegradable Polymer Surfaces." 25(1), 109-110,
5 1998. Controlled Release Society, Inc. Proceed. Int'l. Symp. Control. Rel. Bioact.
6 Mater. 1998]. In this case the binding of biotin to the protein avidin which is very
7 specific is utilized. Biotin is anchored on a polymer surface and biotin is also bound
8 to the substance with which the surface is to be coated. In the presence of avidin,
9 which has several binding points for biotin, the targeted adhesion of the biotinylated
10 compound to the surface then results.

11
12 An advantage of the process is that patterns may be generated on the polymer
13 surface. This is important for tissue where a structured arrangement of cells is
14 necessary.

15
16 However, a disadvantage is that by anchoring avidin, a protein is used which
17 is exogenous and can therefore lead to undesirable reactions. In addition, the
18 substance to be anchored must first be biotinylated, which complicates the process and
19 thus restricts applicability. At the same time, the surface is coated with avidin, which
20 is undesirable for many applications.

21
22 Other methods use a further polymer to adhere surface-modifying substances
23 to the surface of the biodegradable polymer.

24
25 Hence, polyethylene glycol is adhered to the surface to be modified, for
26 example, which assumes the corresponding existence of functional groups to the
27 surfaces [U.S. Patent No. 5,908,828]. In these developments, these functional
28 groups must first be generated in some cases by chemical reactions. This is an

1 additional process step and undesirably increases the expense for application of this
2 process.

3
4 The anchoring of special peptide sequences on ceramics, polyhydroxy ethyl
5 methacrylate and polyethylene terephthalate is described in US Patent 5,330,911. The
6 process assumes the existence of functional groups and is not suitable for the
7 suppression of non-specific adsorption.

8
9 ——— A ~~U.S. Patent No. 5,308,641~~ discloses a further process is based on
10 polyalkylimine as spacer between the polymer surface and the substance to be adhered
11 ~~{US Patent 5,308,641}~~. The process has the same disadvantages as described for
12 ~~US Patent No. 5,330,911~~ and assumes the existence of corresponding
13 functional groups on the polymer surface.

14
15 ——— A process is described in US ~~U.S. Patent 5897955~~ No. 5,897,955 and WO
16 97/46267 A1, ~~disclose a process~~ wherein the surface of the polymer to be modified
17 is firstly coated with a surfactant, which then only after cross-linking forms the actual
18 surface onto which the substances can be bound. The resulting disadvantage here is
19 also that no adequate masking of the surface is achieved and non-specific adsorption
20 cannot be suppressed.

21
22 To increase the compatibility of polymer surfaces, it has been suggested that
23 asymmetric molecules should be bonded onto these surfaces via radical mechanisms.
24 This procedure is therefore bound to specific materials which firstly adsorb on the
25 polymer surface and can then be cross-linked.

26
27 According to the US Patent 5,263,992, the surface of biomaterials is firstly
28 covered with a binding molecule in a radical reaction, in which case the binding
29 molecule carries a functional group, onto which surface-modifying substances are

1 bonded. The disadvantage of the process is again that the adsorption of undesirable
2 substances is not suppressed by this structure.

3
4 US Patent 5,320,840 describes a polymer which is water-soluble and does not
5 therefore meet the requirements for a solid water-insoluble biodegradable matrix.
6 Many processes such as the one described in US Patent 5,240,747, for example,
7 require drastic conditions for the modification of polymer surfaces, e.g. such as
8 radiation with uv light or the presence of functional groups in the form of amino
9 groups or polyamines (US Patent 5,399,665 and US Patent 5,049,403).

10
11 EP 0 844 269 discloses a block polymer with functional groups at both ends,
12 wherein the block polymer is composed from hydrophobic and hydrophilic blocks.
13 The hydrophilic blocks in this case carry as functional groups amino, carboxyl or
14 mercapto groups, which have to be firstly activated for a covalent linkage of surface-
15 modifying molecules of interest, which generally have amino, mercapto, hydroxyl
16 groups or double bonds as functional groups.

17 WO 95/03356 discloses non-linear block copolymers which are composed
18 from a multifunctional polymer, to which hydrophilic and hydrophobic polymers are
19 bonded. In this case a possible covalent bonding of modifying substances is likewise
20 achieved via a terminal hydroxyl group of the hydrophilic block, e.g. of polyethylene
21 glycol, which requires previous activation.

22 23 SUMMARY

24 The examples outlined above show the need for biodegradable polymers which
25 have the following properties:

26
27 1. Adequate masking of the polymer surface for the suppression of non-
28 specific adsorption of substances;

29

- 1 2. Suppression of non-specific adhesion of living cells;
2
- 3 3. Full biodegradability and biocompatibility of the degradation products;
4
- 5 4. Adjustability of the concentration of functional groups on the polymer
6 surface, which are suitable for the chemical reactions with a plurality of surface-
7 modifying substances;
8
- 9 5. Provision of the possibility of coating the polymer surface with several
10 different substances;
11
- 12 6. to permit binding of the surface-modifying substances before or after
13 processing to shaped bodies (e.g. films, porous sponges, microparticles, nanoparticles,
14 micelles etc.), and
15
- 16 7. Formation of patterns by binding surface-modifying substances on the
17 polymer surface.
18

19 Two preconditions must be met in order to permanently anchor surface-
20 modifying substances on polymer surfaces:
21

- 22 1. On their surface the polymers must carry functional groups to which
23 the substances may be chemically bonded.
24
 - 25 2. The functional groups must be readily accessible for these chemical
26 reactions.
27
- 28

While known biodegradable polymers such as poly(~~α-hydroxyesters~~
~~hydroxyesters~~) [e.g. poly(lactide), poly(lactide-co-glycolide)], polyanhydrides or
poly(~~β-hydroxyesters~~~~β-hydroxyesters~~) have suitable functional groups at both
molecule ends, these groups are only accessed on the surface with difficulty.
Poly(lactide), for example, has an alcohol and a carboxylic acid function as end group
which do not, however, permit binding to the polymer surface for the reasons given
above.

To achieve the aforementioned objects, a block copolymer is provided
according to the ~~invention~~~~disclosure~~ containing

a hydrophobic biodegradable polymer ~~a~~,

a hydrophilic biocompatible polymer ~~b~~,

at least one reactive group ~~c~~ for covalent binding of a surface-modifying
substance d) to the hydrophilic polymer b),

wherein the at least one reactive group c) is ~~selected from 1) a functional group~~
~~and/or 2) an at least bifunctional molecule with at least one free functional group with~~
~~the provision that if the hydrophilic component b) is polyethylene glycol, the reactive~~
~~group c) is not a hydroxyl group.~~

According to a further aspect, the ~~invention~~~~disclosure~~ relates to a surface-
modified block copolymer which has as additional component a surface-modifying
substance d) bonded by means of the reactive group c) as binding link, and a process
for the production thereof.

1 In a preferred configuration, the block copolymers are present as shaped
2 bodies.

3
4 The ~~invention~~disclosure further relates to the application of the block
5 copolymers in particular in the field of drug delivery, drug targeting, and preferably
6 for tissue engineering.

7
8 According to a further aspect the disclosure relates to a process for the
9 production of a block copolymer, wherein the binding of the at least one substance d)
10 to the surface of the block copolymer is achieved by generating a substrate pattern,
11 and the reactive group c) is selected from 1) an at least bifunctional molecule with at
12 least one free functional group and/or 2) a functional group, and block copolymers
13 obtainable with this.

14
15 Because of their structure comprising a hydrophobic and a hydrophilic
16 component, the block copolymers according to the ~~invention~~disclosure have a
17 surfactant-like character. This causes the polymer, e.g. upon contact with an aqueous
18 medium, to be subject to an orientation wherein the hydrophilic component b) is
19 present in enriched form on the polymer surface, and thus allows free accessibility of
20 surface-modifying substances d) to the reactive group c) for binding.


21
22 Therefore, the ~~invention~~disclosure relates to polymers, in which a part of the
23 chain, the hydrophilic component b), projects out of the polymer surface and ensures
24 an adequate distance between the polymer surface and reactive group c), as a result
25 of which the binding of surface-modifying substances to the reactive group c) is
26 facilitated.

27

1 As a result, special surfaces may be constructed by simple means and prepared
2 for such applications in the best possible way in which the surface of materials serves
3 to assume a specific functionality.

4
5 At the same time, the block copolymers according to the invention disclosure
6 ensure suppression of the non-specific adsorption of molecules and adhesion of cells
7 to their surface.

8
9 An important property of the block copolymers described here is the full
10 biocompatibility of the molecule parts used, in which case at least the hydrophobic
11 component a) is biologically degradable.

12 
13 These polymers also have an advantage in this respect in comparison to
14 systems already described for the modification of surfaces which make use of
15 polystyrene, glass or metals, for example. [Mikulec, L.J. and Puleo, D.A. J. Biomed.
16 Mater. Res. 32 (1996) 203-208; Puleo, D.A. J. Biomed. Mater. Res. 29 (1995) 951-
17 957; Puleo, D.A. Biomaterials 17 (1996) 217-222; Puleo, D.A. J. Biomed. Mater. Res.
18 37 (1997) 222-228).

19
20 In contrast to the named materials, after implantation into the human or animal
21 body, the block copolymers according to the invention disclosure have the potential
22 to degrade in a specific period of time, depending on the requirement, and to leave the
23 body.

24
25 The material properties of the block copolymer can be fixed by the selection
26 of components a) and b) of the block copolymer, i.e. the type and length of the
27 hydrophobic and the hydrophilic polymer chain. [For example, the mobility of the
28 fixed substance d) can be varied via the length or structure of the hydrophilic
29 component b). The degradation properties, the mechanical strength and the solubility,

for example, in water or an organic solvent of the copolymer can be controlled via the length and structure of the hydrophobic component a).

□

Hence, by changing the biodegradable lipophilic chain of component a) of the block copolymer, it is possible to increase the period of degradation and increase the mechanical strength of the polymers.

The configuration as block copolymer according to the inventiondisclosure supports the orientation, wherein the hydrophilic component predominantly comes to lie on the polymer surface and, for example, promotes the formation of micelles in the aqueous medium.

Figure

BRIEF DESCRIPTION OF THE DRAWINGS

In the drawings:

FIG. 1 shows the binding of a surface-modifying substance d) onto the surface of a block copolymer according to the inventiondisclosure via the reactive group c);

Figure

FIG. 2 shows—□ the structure of a block copolymer according to the inventiondisclosure;

Figure FIG. 3 shows—□ a surface of a block copolymer according to the inventiondisclosure coated with different substances d);

Figure FIG. 4 shows—□ images taken by scanning microscope of block copolymers according to the inventiondisclosure containing different amounts of polyethylene glycol with a molecular weight of 5000 Da and a reference polymer with no PEG;

1 **Figure**

2 **FIG. 5** shows—ESCA spectra of protein adsorption on different polymer films;

3

4 **Figure**

5 **FIG. 6** shows—ESCA spectra of peptide adsorption on different polymer
6 films;

7

8 **Figure**

9 **FIG. 7** shows—images taken by optical microscope of pre-adipocytes 3T3-L1
10 on different polymer films;

11

12 **Figure**

13 **FIG. 8** shows REM images of mesenchymal stem cells from rats on different
14 polymers;

15

16 **Figure**

17 **FIG. 9** shows—determination of the activity of a block copolymer
18 according to the inventiondisclosure via the binding of EDANS, and

19

20 **Figure FIG. 10** shows the binding of trypsin to a polymer according to the
21 inventiondisclosure.

22

23 **DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS**

24 The subscript indexes used in the polymer designations in the figuresFIGS.
25 relate to the molecular weight (Mn) expressed in kDa.

26

27 **Figure**

28 **FIG. 2** shows a surface-modified block copolymer according to the
29 inventiondisclosure with its essential structural elements, hydrophobic component a),

1 hydrophilic component b) and reactive group c) as well as surface-modifying
2 substance d).

3

4 In this case, the hydrophobic component a) serves as carrier and for fixing the
5 entire block copolymer, the hydrophilic component b) serves to make available the
6 reactive group c) for the covalent binding of a surface-modifying substance d) and for
7 masking the surface, and the reactive group c) serves as binding link for the permanent
8 binding of the surface-modifying substance d).

9

10 The block copolymer according to the ~~invention~~disclosure can be brought into
11 any desired suitable shape for the respective applications, the shaped bodies obtained
12 in this case likewise being subject of the ~~invention~~disclosure.

13

14 The block copolymer can, for example, be provided as a film, particle in the
15 desired size, e.g. nano- or micro-particle, or three-dimensional shaped body, e.g.
16 monolith. The shaped bodies can be porous. According to a preferred embodiment,
17 the block copolymer forms a porous shaped body in the manner of a sponge, for
18 example.

19

20 It is advantageous according to the ~~invention~~disclosure that the block
21 copolymer or the shaped bodies formed therefrom are suitable for "instant reactions"
22 with the substance d), which means that they can be produced in advance as stock and
23 stored without problem until application without having to be freshly prepared first
24 for the scheduled application in a time-consuming manner.

25

26 The block copolymer can be composed from one or more, also different,
27 blocks comprising the hydrophobic a) and hydrophilic component b), in which case
28 the individual blocks can contain the same monomers possibly with different chain
29 lengths, or different monomers.

1 According to a preferred configuration, a diblock copolymer is used as block
2 copolymer.

3
4 Components a) and b), simultaneously or independently of one another, can
5 be linear or branched, comb- or star-shaped.

6
7 Component c) can also be a cross-linked compound, if required.

8
9 The surface of the block copolymer can be coated with a single substance or
10 different substances d), the at least one substance d) can form any desired pattern on
11 the surface, e.g. the concentration of the at least one substance d) can be locally
12 constant or variable, it can form a gradient etc.

13
14 The type of coating of the surface can be selected in accordance with the
15 application case. Hence, it has been shown that a gradual coating with growth factors
16 can be advantageous.

17
18 Any biodegradable hydrophobic polymer known for the named applications
19 can be used as biodegradable hydrophobic component a), like those which have
20 already been specified above. Further polymers can be derived from the literature.

21
22 The polymer for component b) can be of synthetic, part-synthetic or natural
23 origin.

24
25 They can be poly(a-hydroxyesters, e.g. polylactic acid, polyglycolic acid and
26 their copolymers), poly(e-caprolactam), poly(b-hydroxyesters (e.g. poly(b-
27 hydroxybutyrate), poly(b-hydroxy valerate))), poly(dioxanon), polymalic acid,
28 polytartaric acid, polyorthoester, polycarbonate, polyamide, polyanhydride,
29 polyphosphazene, peptide, polysaccharide, protein and other polymers such as those

described in Göpferich A. "Mechanism of Polymer Degradation and Elimination" in: Domb A, Kost J, Wiseman D, eds. Handbook of Biodegradable Polymers. Harwood acad. publ. Inc., 1997: 451-472; Göpferich A: "Mechanisms of Polymer Degradation and Erosion" Biomaterials 17 1996a pp. 103-114 and Göpferich A: Biomaterials 17 (1996a) 103-114; Göpferich A., Eur. J. Pharm. Biopharm. 42 (1996b) 1-11; Leenslag, J.W. et al Biomaterials (1987) 311-314; Park, K et al. Biodegradable Hydrogels for Drug Delivery (1993); Suggs, L.J. and Mikos, A.G. (1996) 616-624.

Further suitable compounds are described, for example, in the Handbook of Biodegradable Polymers (1997) 451-472.

The hydrophobic polymer a) is preferably at least one polymer selected from a polyester, poly-ε-caprolactam, poly-α-hydroxyester, poly-β-hydroxyester, polyanhydride, polyamide, polyphosphazene, polydioxanon, polymalic acid, polytartaric acid, polyorthoester, polycarbonate, polysaccharide, peptide and protein.

The hydrophobic polymer a) is, in particular, at least one polymer selected from polylactide, polyglycolide, poly(lactide-co-glycolide), poly-β-hydroxybutyrate and poly-β-hydroxyvalerate.

The hydrophobic component a) is preferably water-insoluble.

The polymers particularly suited as biodegradable component a) are those in which the polymer chain degradation can be brought about by hydrolysis, enzymatic, photolytic or other reactions.

The minimum chain length n measured in monomers amounts to n=2, the upper limit results from the maximum achievable molar masses for the respective

1 monomer in the polymerisation reaction or from the desired properties for the
2 polymer, i.e. depending on the intended application.

3
4 As part of the present ~~invention~~disclosure the details concerning the molar
5 masses (molecular weight), unless specified otherwise, relate to the numerical mean
6 Mn.

7
8 Hence, the chain length of the polymers for component a) can move from few
9 to several thousand monomer units and the polymer can have a molecular weight of
10 over 10 million Dalton.

11
12 For example, for polylactide an upper limit of the molar mass of up to 100 000
13 Da is preferred.

14
15 As already mentioned above, the length of the hydrophobic component a)
16 determines the properties of the block copolymer such as the degradation properties
17 and the mechanical strength.

18
19 For example, in the case of a combination preferred according to the
20 ~~invention~~disclosure of poly(D,L-lactide) (PLA) as hydrophobic component a) and
21 poly(ethylene glycol) (PEG) for the hydrophilic component b), a chain length of the
22 hydrophobic component a) of approx. $n < 20$ leads to water-soluble products. If the
23 PEG content is greater than the PLA content, then water-soluble products can likewise
24 be expected.

25
26 A synthetic, part-synthetic or natural biocompatible hydrophilic polymer,
27 which can also be biologically degradable, may be used as hydrophilic component b).

28

1 It is built up from at least bifunctional and preferably water-soluble structural
2 elements.

3
4 Examples of suitable polymers are polyethylene glycols, polyacrylamides,
5 polyvinyl alcohol, polysaccharides (e.g. modified celluloses and starches), alginates,
6 peptides and proteins.

7
8 Preferred examples for the hydrophilic component b) are polyethylene glycol,
9 polypropylene glycol, polyethylene glycol/polypropylene glycol copolymer,
10 polyethylene glycol/polypropylene glycol/polyethylene glycol copolymer,
11 polybutylene glycol, polyacrylamide, polyvinyl alcohol, polysaccharide, peptide and
12 protein.

13
14 If a symmetric molecule such as PEG, for example, with two like functional
15 end groups, in this case hydroxyl, is used as hydrophilic component b), it should be
16 ensured during linkage with the hydrophobic component a) that the hydrophobic
17 component does not react with both functional end groups simultaneously, and thus
18 none of the functional end groups remains available as reactive group c) for the
19 covalent binding of surface-modifying substances.

20
21 To avoid this problem, a hydrophilic component b) with two different
22 functional end groups is used for the synthesis, as will be explained below by the
23 example of the preferably used PEG, in which case these explanations apply
24 analogously for other symmetric molecules which may be used as hydrophilic
25 component b) for the block copolymer according to the invention. Thus,
26 in the case of PEG with two hydroxyl groups as end groups, one of the hydroxyl
27 groups is replaced by another functional group.

28

1 For example, poly(ethylene glycol) amine (PEG-NH₂) may be used, in which
2 case an end hydroxyl group is replaced by a primary amino group.

3 □
4 This permits the adhesion of the monomers of the hydrophobic component a)
5 to be controlled as part of the synthesis in such a way that the chemical reaction only
6 proceeds at one molecule end.

7
8 The type of functional end groups is not restricted in this case to hydroxyl
9 groups and amino groups. Alternatively, thiol groups, double bonds or carbonyl
10 functions may be used for synthesis. Further functional groups are known per se and
11 can be derived from the literature.

12
13 The chain length of the hydrophilic component is also determined in
14 accordance with the application and requirement.

15
16 For example, the minimum chain length for PEG or of an asymmetric
17 substituted PEG such as PEG-NH₂, for example, is at an ethylene
18 unit (ethanolamine).

19
20 The upper limit can be set for specific applications in human and animal
21 bodies by the requirement that the released fragments should still be capable of
22 passing through the kidneys and can be excreted.

23
24 Suitable molar masses preferably lie at 200 to 10⁵ Da, particularly
25 preferred at 1³/₂000 to 10⁴/₃000 Da, ~~whereas in which case~~, in particular for applications
26 outside a human or animal body, polymers with higher molar masses of up to several
27 million Da may also be used.

28

1 Above all, PEG has proved to be particularly suitable to masking a polymer
2 surface against the adsorption of molecules and the adhesion of cells.

3
4 Block copolymers composed from the following combinations are particularly
5 preferred according to the invention disclosure.

6
7 The hydrophobic polymer a) is at least one selected from polylactide,
8 polyglycolide, poly(lactide-co-glycolide).

9
10 Particularly preferred is a polylactide, e.g. a poly(D,L-lactide), preferably with
11 a molar mass in a range from 1-³/₂000 to 100-³/₂000, in particular up to 50-³/₂000 Da.

12
13 The hydrophilic polymer b) is a polyethylene glycol (PEG), whereby in
14 polyethylene glycols with a molar mass in a range from 200 to 10-³/₂000 Da, in
15 particular 1-³/₂000 to 10-³/₂000 Da, are particularly preferred.

16
17 In principle, the reactive group c) can be any desired functional group or an
18 at least bifunctional molecule, which can form a covalent bond with the selected
19 surface-modifying substance d), with the provision that an at least bifunctional
20 molecule is used as reactive group c) for a block copolymer according to one of
21 Claims 1 to 19.

22
23 The reactive group c) can comprise:

24
25 a single functional group (e.g. amino group, carboxyl group) and thus direct
26 activation of the hydrophilic polymer (e.g. activated acid function or epoxide);

27
28 physiological dicarboxylic acids (succinic acid, tartaric acid and variants
29 thereof such as those described in Anderson, G.W. et al. J.Am.Chem.Soc.86 (1964)

1839-1842), which are provided with terminal groups (succinimidyl esters) in order to achieve the formation of one or two acid amide groupings;

dialdehydes (e.g. glutaric dialdehyde);

special "molecules" for the selective binding of thiols such as those described in Hermanson, G.T. Bioconjugate Techniques (1996), e.g. N-succinimidyl-3-(2-pyridyldithio)propionate (SPDP) or succinimidyl-4-(N-maleimidomethyl)-cyclohexane-1-carboxylate (SMCC);

photoreactive crosslinkers such as those described in Hermanson, G.T. Bioconjugate Techniques (1996), e.g. N-hydroxysuccinimidyl-4-acidosalicylic acid (NHS-ASA), sulphosuccinimidyl-2-(p-acidosalicylic amido)ethyl-1,3'-dithiopropionate (SASD);

splittable crosslinkers such as those described in Hermanson, G.T. Bioconjugate Techniques (1996), e.g. compounds from the above-mentioned groups, which may be split by special reagents e.g. disulphides by hydrogenolysis or by disulphide exchange, glycol groups with periodate (e.g. in the case of tartaric acid), ester groups with hydroxylamine; and

enzymatically splittable molecules such as corresponding peptides, e.g. the sequence Leu-Gly-Pro-Ala, which can be split from collagenase, or oligosaccharides.

Particularly preferred examples of reactive groups c) are those selected from at least one amino group, hydroxyl group, thiol,-carboxylic acid, acid chloride, keto group, - and in particular for the subject of Claims 1 to 19 - dicarboxylic acid amide, 3-maleic imidopropionic acid-N-succinimidyl ester and succinimidyl ester.

1 ~~In the case of PEG, which is a symmetric compound, the reactive group c) selected~~
2 ~~should differ from hydroxyl.~~

3
4 In principle, the synthesis of the block copolymer according to the
5 ~~invention disclosure~~ may be achieved in various ways, in which case conventional
6 methods of polymer chemistry are used.

7
8 On the one hand, the blocks a) and b) can be synthesised separately and
9 subsequently bonded covalently. Alternatively thereto, it is possible to present a
10 polymer chain and synthesise the missing chain by polymerisation at a polymer chain
11 end. Hence, it is possible, for example, to synthesise block copolymers from
12 poly(D,L-lactide) and poly(ethylene glycol) amine (PLA-PEG-NH₂) by presenting
13 PEG-NH₂ and synthesizing the biodegradable PLA chain by ring-opening
14 polymerisation from dilactide on the hydroxy end of the PEG-NH₂. In principle, the
15 reverse procedure is also possible.

16
17 In this case, the reactive group c) can already be present in the polymer
18 obtained, as in the above example, or a functional group present in the hydrophilic
19 component b) can be converted or introduced, where needed, for binding the desired
20 surface-modifying substance d) to a suitable reactive group c).

21
22 Hence, the block copolymer can be modified with nucleophilic groups by
23 coupling an at least bifunctional molecule, e.g. disuccinimidyl succinate, to a free end
24 group of component b).

25
26 In the simplest case, this reaction can take place in solution, DMSO, for
27 example, is suitable as solvent in the case of ~~PLA-PEG-NH₂PLA-NH₂~~. After
28 preparation of the block copolymer, e.g. to form a suitable shaped body, the reaction
29 can also take place on the surface thereof.

1 The advantage of activation with a reactive group c) is that the linking of many
2 surface-modifying substances d) proceeds in water. As a result of the reactive group
3 c), which is linked to the hydrophilic block b), this block ends with an active group,
4 which is capable of binding other molecules with nucleophilic functional groups, such
5 as amino groups, for example. ~~Figure~~~~FIG.~~ 1 schematically shows the adhesion of a
6 surface-modifying substance to such a polymer surface.

7
8 The desired surface property can then be set via the subsequently occurring
9 adhesion of the surface-modifying substance d) to the hydrophilic molecule part b).

10
11 Surface-modifying substances d), which may be used for a bond, are generally
12 those carrying a nucleophilic group - e.g. an amino group -, such as carbohydrates, for
13 example, including amongst others: mono-, oligo-, and polysaccharides and
14 glycosides, peptides, proteins, heteroglycans, proteoglycans, glycoproteins, amino
15 acids, fats, phospholipids, glycolipids, lipoproteins, medicinal agents, antibodies,
16 enzymes, DNA/RNA, cells, which can bond directly, for example, via proteins located
17 on the cell membrane, but also dyes and molecular sensors.

18
19 Examples for peptides are those with the motif -RGD-, IKVAV or YIGSR and
20 for proteins growth factors, e.g. IGF, EGF, TGF, BMP and basic FGF, proteins and
21 glycoproteins of the extracellular matrix such as fibronectin, collagen, laminin, bone
22 sialo protein and hyaluronic acid. Further substances are described in the relevant
23 literature.

24
25 The block copolymer according to the ~~invention~~~~disclosure~~ is particularly
26 suitable for the production of drug targeting systems, drug delivery systems,
27 bioreactors, preferably porous shaped bodies,
28
29 for therapeutic and diagnostic purposes, for tissue engineering and as emulsifier.

1 The binding of the surface-modifying substance is explained in more detail
2 below, in general terms and with respect to preferred applications.

3

4 For the binding, the block copolymer, like the substance, can be present in
5 solution or the block copolymer forms an immobilized solid surface, to which binds
6 the substance d) present in solution.

7 In this case, a decisive advantage of the use of the block copolymer according
8 to the invention disclosure is that under very mild conditions the linking reactions may
9 also be conducted in aqueous medium and therefore sensitive substances d) may also
10 be bonded in.

11

12 Hence, proteins can be fixed at room temperature and with a pH suitable for
13 the protein without being denatured on the polymer surface. Alternatively, substances,
14 which are to be bonded to the surface by means of light radiation, can be dissolved in
15 any desired solvent in which the polymer is insoluble. Upon subsequent radiation with
16 uv light, the binding to the surface can then also be linked at room temperature.

17

18 Therefore, several conditions are conceivable, in principle, for a binding
19 process, wherein by using the block copolymer according to the invention disclosure
20 there is sufficient freedom to select optimum conditions with respect to the stability
21 of the substance d) and the polymer.

22

23 As a result of the simple type of binding of also unchanged, i.e. non-activated
24 substances d), to the block copolymer with reactive group c) made possible according
25 to the invention disclosure, the process can be simplified insofar as it is only necessary
26 to dip the finished preshaped polymer carrier, e.g. in the form of micelles, nano-
27 particles, polymer film or polymer sponge, into the solution of substance d) in order
28 to then obtain the finished modified system after a predetermined reaction period
29 (instant reaction).

1 However, alternatively to the described binding of substance d) to the polymer
2 with reactive group c), the other way round is also possible, namely to first activate
3 the substance d) to be bound with the reactive substance c) for a bond, and then bind
4 the complex comprising substance d) and reactive group c) via the reactive group c)
5 to the component b) of the block copolymer comprising a) and b) to form the finished
6 surface-modified block copolymer according to the inventiondisclosure.

7
8 However, a disadvantage in this case is that a larger excess of the reactive
9 group c), e.g. a low-molecular dicarboxylic acid here, is generally necessary for
10 activation of the substance d) by binding the reactive group c) in order to prevent the
11 formation of dimers. However, this must be removed again after activation. The
12 consequence of this is, above all with likewise low-molecular substances d), that the
13 purification is more difficult to configure.

14
15 In addition to the production of homogeneously coated surfaces, non-
16 homogeneously coated surfaces may also be easily produced with the block
17 copolymers according to the inventiondisclosure. This means that, for example,
18 gradients or patterns of the surface-modifying substances d) can also be generated on
19 these polymers. This can be achieved by spot application of the substances d) (e.g.
20 using an ink jet process) or by spot activation of the reactive groups c) by radiation
21 (e.g. with uv light), bombardment with particles, stamping or soft lithography.

22 Hence, structured surfaces can be formed which also allow any desired
23 combinations of substances d) to be examined for their effect on cells, for example,
24 or to cultivate combinations of cells in very special spatial orientation to one another
25 or also to construct miniature biotechnological factories using enzymes which perform
26 special reactions in a linked process. FigureFIG. 3 shows such surfaces which are
27 distinguished by two different substances d) and additionally also an inert shorter
28 component.

29

1 As part of tissue engineering, it is possible to influence the adhesion,
2 proliferation and differentiation of cells in a better way than previously, since the
3 block copolymers according to the invention enable an exact coating of the
4 surface with one or more substances d). At the same time, the non-specific interaction
5 of unwanted substances d), in particular unwanted cells, is suppressed with the
6 polymer surfaces.

7
8 As part of drug delivery, it is possible to use the polymers for surface
9 modification, which distributes small polymer particles to specific tissues or organs
10 (drug targeting). This is achieved by binding specific substances d) such as plasma
11 proteins, antibodies or lectins, for example. Further substances d) possible for this are
12 described in the relevant literature.

13
14 A further application lies in the chemical bonding of polymers in the form of
15 particles to tissue (bioadhesive systems). An active substance can be distributed in
16 increased concentration to the target tissue by this application.

17
18 As a result of the polymer degradation it is to be expected that the substance
19 d) adhered to the polymer block b) is released as part of the hydrolysis. This dynamic
20 process permits the time

1 controlled change of the surface properties of the block copolymer according to the
2 invention disclosure.

3
4 The polymers according to the invention disclosure may also be used for
5 diagnostic purposes by binding substances d) to their surface, which form a bond with
6 the molecules to be analyzed. The analyzed product can then be separated from the
7 sample together with the polymer (e.g. via a suitable shaped body).

8
9
10 The production of a block copolymer according to the invention disclosure as
11 well as the subsequent binding of a protein is illustrated below using the example of
12 PEG-PLA to explain the invention disclosure in more detail.

13 WORKING EXAMPLES

14 EXAMPLE 1. Example: Production of NH₂-PEG-PLA

15 PRODUCTION OF NH₂-PEG-PLA

16 a) Synthesis of NH₂-PEG

17
18
19 Production was conducted in accordance with Yokohama, M. et al. Bioconj. Chem.
20 3 (1992) 275-276.

21
22 The desired amount of ethylene oxide was passed into dry THF in a three-
23 necked flask at -79°C (dry ice + methanol bath) and dissolved therein. The ethylene
24 oxide bottle was weighed after introduction, and thus the presented amount of
25 ethylene oxide was determined. In accordance with the desired molecular weight of
26 the polymer, the calculated amount of 0.5M solution of potassium-bis-(trimethylsilyl)
27 amide in toluene was then added from a dropping funnel.

The reaction mixture was then stirred in the closed three-necked flask at 20°C for 36 hours. The polymer solution thus obtained was dropped into the 12-fold amount of ether, and the precipitated polymer was filtered out. After the polymer obtained was dissolved in THF, a small amount of 0.1N hydrochloric acid was added and the silylamide was thus split. The solution of the finished end product thus obtained was stirred for 5 minutes at room temperature and once again passed into ether in order to precipitate the pure polymer.

b) Synthesis of NH₂-PEG-PLA

Synthesis was conducted in accordance with Kricheldorf, H.R. and Kreiser-Saunders, I. *Macromol. Symp.* 103 (1996) 85-102; Leenslag, J.W. and Pennings, A.J. *Makromol. Chem.* 188 (1987) 1809-1814.

The starting products of the synthesis: the NH₂-PEG synthesized in accordance with 1a) and cyclic DL-dilactide (3,6-dimethyl-1,4-dioxan-2,5-dione), were each passed into a round flask in the desired weight proportions and dissolved in A.R. toluene. For this, the two flasks were heated at the water separator in order to remove the water still present in the toluene. The solutions thus obtained were then combined in the three-necked flask and once again heated in a permanent nitrogen flow.

The weighed catalyst (tin-2-ethylhexanoate) was then added to the boiling reaction mixture and the mixture was then kept boiling for 8 hours.

The polymer solution thus obtained was passed into a round flask after cooling and rotated three times with dichloromethane in the rotary evaporator until dry. After rotating twice after the addition of acetone, the polymer thus obtained was once again dissolved in acetone and dropped into ice-cooled demineralized water and precipitated thereby. The polymer threads thus obtained were separated through a filter and passed into a vacuum drying cupboard. Determination of the molecular mass can be performed by GPC.

1 c) Synthesis of the disuccinimidylester of tartaric acid (DSWS)

2 ¶ Synthesis was conducted in accordance with Anderson, G.W. et al. J. Am. Chem.
3 Soc. 85 (1964) 1839-1842.

4 The calculated amounts of tartaric acid and N-hydroxy succinimide were
5 dissolved in a round flask in a mixture comprising dioxan and ethyl acetate (4:1). ¶ To
6 this solution the solution of the catalyst (dicyclohexylcarbodiimide) was added in the
7 same solvent mixture and the whole was stirred in an ice bath at 0°C for 20 hours.
8 The precipitate thus obtained was filtered off and washed with dioxan. ¶ The end
9 product was extracted from this precipitate by careful heating with acetonitrile. ¶ The
10 solution thus obtained was concentrated to low volume in the rotary evaporator and
11 the product dried in the vacuum cupboard.

12

13 d) Synthesis of SWS-NH-PEG-PLA

14 ¶ The starting products obtained in accordance with 1c) and 1b): disuccinimidyl
15 tartaric acid and NH₂-PEG-PLA, were dissolved in acetonitrile with a slight excess of
16 the diester and provided with a few drops of triethylamine. ¶ After brief heating to
17 boiling, the mixture was stirred for 24 hours. ¶ The end product was separated from the
18 acetonitrile by rotation and dissolved in acetone. ¶ The polymer solution thus obtained
19 was dropped into water and the precipitate filtered off. ¶ The finished active polymer
20 was available after drying in the vacuum.

21

22 According to the above-described procedure NH₂-PEG-PLA diblock
23 copolymers according to the invention disclosure were produced with different
24 molecular masses for the components a) and b) for the subsequent experiments or
25 polymers inactivated analogously with methyl groups, in which the reactive group c)
26 was replaced by a methyl group.

27

28

29

ExampleEXAMPLE 2

Production of amino-polyethylene glycol-poly-L-lactide (NH₂-PEG-PLLA)

The procedure was essentially as in Example 1b).

However, cyclic L-dilactide was used instead of the cyclic D,L-dilactide.

Further, after rotation three times with dichloromethane, the polymer obtained was once again dissolved in dichloromethane and dropped into ice-cooled diethylether. The polymer thread thus obtained were separated through a filter and passed into a vacuum drying cupboard for drying.

Determination of the molecular weight was achieved by GPC and determination of the numerical mean molecular weight was also achieved by ¹H-NMR via calculation of the integrals.

ExampleEXAMPLE 3

Linkage of surface-modifying substances d)

Binding of surface-modifying substances can be conducted in accordance with the processes described in Hill, M. et al. FEBS Lett. 102 (1979) 282-286; Schulman, L.H. et al. Nucleic Acids Res. 9 (1981) 1203-1217.

The linkage of surface-modifying substances d) to the block copolymer according to the inventiondisclosure obtained in accordance with Example 1 can occur in two ways, in principle. Firstly, it is possible to bind the substance d) and the block copolymer in solution if the substance d) passes through the subsequent processing steps undamaged. Alternatively, the block copolymer may firstly be processed to the desired form and the substance d) is then linked. In both cases, it should be assured by buffering that an amino group, for example, is present in unprotonated form in order to obtain quantitative yields where possible. Moreover,

with buffering the location of the bond to the substance d) can still be controlled if the pH is selected so that only an amino group is present in unprotonated form, for example.

ExampleEXAMPLE 4

Characterization of polymer films - properties of the block copolymers

4a) Examination of the block copolymers with AFM

Scanning microscopy was used to characterize the surface topography of the block copolymers according to the inventiondisclosure. For this, the polymers were applied in a 5% solution in chloroform to small square metal plates (5x5 mm) by means of spincoating and then dried. The films thus obtained were then examined with AFM.

The results are shown in FigureFIG 4:

What are obtained are different concentrations, depending on the polymer examined, of humped raised portions on the polymer surface. The raised portions are crystallites of the polyethylene glycol which increase with the increasing content of polyethylene glycol in the block copolymer. This means that the polymers are distinguished by a phase separation of the blocks and thus an availability of the hydrophilic chains on the polymer surface.

4b) Examination of the protein adsorption

Examination of the protein adsorption and its suppression was conducted on different PEG-PLA block copolymers according to the inventiondisclosure, which contained a methyl group in place of a reactive group c) and were thus inactivated for the protein bonding.

For examination of the adsorption of proteins onto the polymer films such inactive polymers were poured out onto small metal plates (0.5x.05 mm) and intensively dried (for at least 2 days in a vacuum), the films thus obtained were then incubated with the protein solutions to be examined and washed off after washing several times with phosphate-buffered (pH=7.4) of isotonic solution. The films thus obtained were then dried again and measured with ESCA.

The model substances were foetal cow serum, atrial natriuretic peptide and salmon calcitonin.

The ESCA spectra served to quantify the adsorbed protein or peptide, since nitrogen was also to be found on the polymer surface as a result of the amino acids of the adsorbed protein. As comparison, polymer films from pure polylactic acid as well as non-incubated polymer films were used.

The results are shown in Figures 5 and 6.

A suppression of the adsorption dependent on the type of surface-modifying substance d) respectively used was observed.

Hence, the adsorption of foetal cow serum was completely suppressed by inclusion of a hydrophilic chain as part of the measurement accuracy (see Figure 5). In the case of the model peptides calcitonin and atrial natriuretic peptide (ANP), a low adsorption of peptide is still identifiable in part (see Figure 6).

Therefore, it was established in the result that the block copolymers according to the invention are able to control the adsorption of proteins and peptides and can therefore have influence on the behaviour of cells which come into contact with the modified polymer surface.

Example 5

Examination of the adhesion behaviour with respect to cells

5a) Cells from a pre-adipocyte cell line were put in a suspension on poured films made of different polymers and their adhesion assessed after 5 hours and 24 hours. For this, the suspensions were washed off with buffer prior to microscopy, and thus only the firmly adhered cells were observed.

The results are shown in Figure FIG. 7.

What is evident are differences in the cell behaviour dependent on which polymers were used. Hence, for example, on the MePEG₅PLA₂₀ no adhered cells can be recognized both after 5 hours and 24 hours, in which case cells are evident on a small scale on the block copolymer MePEG₅PLA₂₀ with the shorter PEG chain, however these adhered only poorly in comparison to the sample composed of lipophilic polylactic acid. After 5 hours only loosely bonded cell aggregates were found and only after 24 hours were single instances of already spread, i.e. firmly bonded, cells found.

However, it can be established in the result that the block copolymers according to the invention can suppress or reduce the adhesion of cells and can thus prevent or restrict the number of non-specific interactions.

5b) For examination of the adhesion of stem cells of rats, thin polymer films made of different block copolymers according to the invention inactivated with methyl (Me-PEG₂-PLA₂₀, Me-PEG₂-PLA₄₀ and Me-PEG₅-PLA₄₅), and for comparison made of PLA, TCPS (tissue culture polystyrene) as well as RG756 (a trade mark for poly(D,L-lactide-co-glycolide 75:25), were poured out on polypropylene discs. The bone marrow stem cells of 6 week old male Sprague Dawley rats with a concentration of 5000 cells per cm³ were cultured onto these films. After 3 hours the morphology of the adhered cells was then observed with the scanning electron microscope.

The results obtained are shown in Figure FIG. 8.

The number of cells was additionally determined by counting using the optical microscope.

1 It was evident that the number of cells on the block copolymer according to the
 2 ~~invention disclosure~~ was less, the larger the hydrophilic component b) of the polymer.
 3 Moreover, the images taken by scanning electron microscope showed that any cells
 4 which had adhered to the block copolymer according to the ~~invention disclosure~~ were
 5 in some cases more rounded than on the reference polymers comprising only
 6 hydrophobic constituents, which is a clear sign for the low adhesion tendency of the
 7 cells to the polymer surface.

8 ~~Example~~ **EXAMPLE 6**

10
 11 Characterization of the active polymers with respect to their binding capabilities

12
 13 6a) Identification of the binding capability with simple model substances with
 14 amino group in solution

15 For examination of the reactivity in solution, a specific amount of polymer
 16 (SWS-NH-PEG₂-PLA₂₀) (50 mg) was dissolved in 2000 µl of dimethylformamide
 17 (DMF) and mixed with a specific amount of dye (EDANS, 5-((2-
 18 aminoethyl)amino)naphthalene-1-sulphonic acid, sodium salt, 0.1-4 mg) which was
 19 also dissolved in DMF. In order to exclude any possible protonation of the amino
 20 group, 20 µl of triethylamine were added as proton catcher. [The solution thus
 21 obtained was then incubated overnight in the agitator at 37°C.] After the reaction
 22 period, 200 µl of the solution were then diluted with 1800 µl of chloroform and the
 23 excess precipitated dye was separated by filtration. 200 µl of the clear solutions were
 24 then measured by means of gel-permeation chromatography. [The amount of
 25 covalently bonded dye was determined via the increase in uv absorption at 335 nm.

26
 27 The result is shown in ~~Figure~~ **FIG. 9**.

1 If the surfaces obtained are evaluated, then a diagram is obtained in which an
2 increase in peak surface may be observed as the amount of dye increases. From a
3 specific amount of dye a plateau is then obtained which is also determined by the
4 restricted number of reactive groups. The amount of reactive groups in a batch of
5 polymer may be simply determined via this determination.

6
7 6b) Identification of the binding capability with simple model substances with
8 amino group on solid polymer surfaces.

9 The activity on solid surfaces may be examined just as the activity in solution.
10 For this, films of an active block copolymer according to the invention disclosure
11 (SWS-NH-PEG₂-PLA₂₀), which had been poured onto round glass cover plates, were
12 coated with an aqueous solution of the dye (5-amino eosin) and this solution was then
13 left to work for two hours. The marked films thus obtained were washed with
14 phosphate buffer several times and then dried. The dried films were then dissolved
15 in chloroform and then separated by means of GPC possibly adsorbed from covalently
16 bonded dye.

17 The presence of an increased UV absorption was observed with the molecular
18 weight of the polymers. This UV absorption may be explained by a covalent bond
19 between dye and polymer.

20
21 6c) Binding of proteins.

22 For examination of the binding ability also of more complex compounds such
23 as proteins, the enzyme trypsin was used as model substance.

24 To bind the enzyme to polymer films, films of the various polymers (SWS-
25 NH-PEG₂-PLA₂₀ with PLA for comparison) poured onto glass cover plates were
26 incubated with solutions of the enzyme trypsin in phosphate-buffered isotonic
27 common salt solution (PBS buffer). The concentrations of the enzyme used for this
28 amounted to 0.5 or 1.0 mg/ml.

1 The polymers linked with trypsin thus obtained, after an incubation period of
2 2 hours, were then washed 3 times with PBS buffer containing 0.05% Tween 20 in
3 order to remove any possibly adsorbed protein as effectively as possible. The films
4 thus washed were then wiped dry and transferred into six-well plates. 2 ml of the
5 reaction medium were then added to each individual well of the plates and the
6 enzymatic reaction was conducted in the incubator for 2 hours at 37°C. The reaction
7 medium was a 1 millimolar solution of benzoyl-L-arginine ethyl ester (BAEE) in tris-
8 buffer with pH=8.0. After 2 hours the enzymatic reaction was stopped by adding an
9 aqueous solution of a trypsin inhibitor composed of soya beans and the transformation
10 of the enzyme substrate was thus terminated. The solutions thus obtained were
11 measured at 253 nm by uv-photometric means.

12
13 The result is shown in Figure FIG 10.

14 The comparison with PLA and with the pure glass cover glasses shows a clear
15 increase in the substrate conversion in the case of the block copolymer according to
16 the invention disclosure which is caused by the amount of covalently bonded enzyme.